Claims

What is claimed is:

1. A method for detecting drug resistance of HIV in a sample, comprising:

taking a culture of recombinant cells in which at least one of the recombinant cells comprises

a reporter sequence comprising a reporter gene whose expression is regulated by a protein specific to HIV,

CD4, and

one or more cell surface co-receptors for HIV, wherein the one or more cell surface co-receptors are each encoded by a heterologous sequence and expressed at an elevated level relative to the level of the corresponding cell surface co-receptor naturally expressed in a human cell such that productive infection of the recombinant cell by HIV is achieved, which is defined by HIV viral replication and the infection of non-infected cells in the culture of the recombinant cells;

contacting the cell culture with a first sample containing HIV;

adding an anti-HIV agent to the cell culture; and

detecting a change in a level of expression of the reporter gene in the cells in the culture.

2. The method according to claim 1, wherein the reporter gene expression is up-regulated by the HIV specific protein.

3. The method according to claim 1, wherein the HIV specific protein is an HIV transactivator protein.

4. The method according to claim 3, wherein the HIV transactivator protein is Tat.

5. The method according to claim 1, wherein the reporter sequence comprises a promoter, an HIV-specific enhancer sequence, and a reporter gene whose expression is regulated by binding of an HIV-specific transactivator protein to the HIV specific enhancer sequence.

6. The method according to claim 5, wherein the HIV specific transactivator protein is

Tat and the HIV-specific enhancer sequence comprises at least one copy of TAR sequence.

7. The method according to claim 6, wherein the HIV-specific enhancer comprises at least two copies of TAR sequence.

8. The method according to claim 1, wherein the reporter gene is selected from the group consisting of genes encoding β-galactosidase, luciferase, β-glucuronidase, chloramphenicol acetyl transferase (CAT), fluorescent protein, secreted embryonic alkaline phosphatase (SEAP), hormones and cytokines.

9. The method according to claim 1, wherein the one or more additional cell surface receptors expressed by the recombinant cell are selected from the group consisting of CXCR4, CCR5, CCR1, CCR2b, CCR3, CCR4, CCR8, CXCR1, CXCR2, CXCR3, CX_3CR1, STRL33/BONZO and GPR15/BOB.

10. The method according to claim 1, wherein the one or more additional cell surface receptors expressed by the recombinant cell comprises CXCR4.

11. The method according to claim 1, wherein the one or more additional cell surface receptors expressed by the recombinant cell comprises CCR5.

12. The method according to claim 1, wherein the one or more additional cell surface receptors expressed by the recombinant cell comprises CXCR4 and CCR5.

13. The method according to claim 1, wherein the recombinant cell expresses a sufficient number of cell surface receptors to render the recombinant cell susceptible to infection of substantially all strains of HIV.

14. The method according to claim 1, wherein the recombinant cell expresses a sufficient number of cell surface receptors to render the recombinant cell susceptible to infection of substantially all subtypes or clades of HIV.

15. The method according to claim 1, wherein the recombinant cell expresses a sufficient number of cell surface receptors to render the recombinant cell susceptible to infection of clinical isolates of HIV.

16. The method according to claim 1, wherein the recombinant cell is a tumor cell.

17. The method according to claim 1, wherein the recombinant cell is a cell which has been immortalized by introducing a gene into the cell which renders the cell line immortalized.

18. The method according to claim 1, wherein the recombinant cell is capable of achieving productive infection of a clinically isolated HIV.

19. The method according to claim 1, wherein the human cell is from a stable human cell line.

20. The method according to claim 19, wherein the human cell line is a human T-lymphoma cell line HUT78.

21. The method according to claim 19, wherein the co-receptor for HIV is CXCR4 or CCR5 and is expressed at a level of at least 2-folds of that of the CXCR4 or CCR5 naturally expressed in a HUT78 cell.

22. The method according to claim 19, wherein the co-receptor for HIV is CXCR4 or CCR5 and is expressed at a level of at least 5-folds of that of the CXCR4 or CCR5 naturally expressed in a HUT78 cell.

23. The method according to claim 1, wherein the human cell is a human peripheral blood cell (PBMC).

24. The method according to claim 1, wherein CD4 receptor and the one or more cell surface co-receptors for HIV are expressed by an adenoviral vector tranduced into the recombinant cell.

25. The method according to claim 24, wherein the adenoviral vector is replication incompetent.

26. The method according to claim 24, wherein the adenoviral vector has 1-100 multiplicity of infection.

27. The method according to claim 24, wherein the adenoviral vector has 10-60

multiplicity of infection.

28. The method according to claim 24, wherein CD4 is expressed from the E1 region of the adenoviral vector while the one or more cell surface co-receptors for HIV are expressed from E3 or E4 region of the adenoviral vector.

29. The method according to claim 24, wherein the one or more cell surface co-receptors for HIV are CCR5 and CXCR4 that are bicistronically expressed from E1, E3, or E4 region of the adenoviral vector by a splicing mechanism or via an internal ribosome entry site.

30. The method according to claim 24, wherein the native E1 promoter of the adenoviral vector is replaced by an exogenous promoter for expressing CD4 or the one or more cell surface co-receptors for HIV.

31. The method according to claim 30, wherein the exogenous promoter is a CMV promoter.

32. The method according to claim 1, wherein the HIV contained in the first sample is a laboratory isolate of HIV.

33. The method according to claim 1, wherein the HIV contained in the first sample is a clinical isolate of HIV.

34. The method according to claim 1, wherein the first sample containing HIV is a blood sample of an individual infected with HIV.

35. The method according to claim 1, wherein the first sample containing HIV is selected from the group consisting of whole blood, blood serum, isolated peripheral blood cells, T cells, spleens, and bone marrow.

36. The method according to claim 1, wherein the HIV contained in the first sample is HIV from a clinical isolate that has been propagated in human blood cells to increase viral titer.

37. The method according to claim 1, wherein the anti-HIV agent is added to the cell culture before the cell culture is contacted with the first sample containing HIV.

38. The method according to claim 1, wherein the anti-HIV agent is selected from the group consisting of consisting of nucleoside RT inhibitors, nonnucleoside RT inhibitors, protease inhibitors, integrase inhibitors, viral protein antagonists, capsid lockers, antisense and ribozyme oligonucleotides against HIV mRNA or viral RNA genome, decoys of TAR sequence and RRE, soluble CD4, Gag and Env protein mutants, viral entry inhibitors and fusion inhibitors.

39. The method according to claim 1, wherein the anti-HIV agent is selected from the group consisting of zidovudine, didanosine, zalcitabine, lamivudine, stavudine, abacavir, nevirapine, delavirdine, efavirenz, indinavir, ritonavir, saquinavir, nelfinavir, and amprenavir.

40. The method according to claim 1, further comprising:

tittering for the number of infectious HIV particles contained in the sample before contacting the cell culture with the first sample.

41. The method according to claim 40, further comprising:

propagating the HIV contained in the sample to increase viral titer before contacting the cell culture with the first sample.

42. The method according to claim 1, further comprising:

repeat the steps in claim 1 for a second sample containing a reference HIV strain, and

comparing the change in the level of expression of the reporter gene for the second sample with that for the first sample, wherein an increase in the expression of the reporter gene in the first sample indicates resistance of HIV contained in the first sample to the treatment with the anti-HIV agent.

43. The method according to claim 42, wherein the reference HIV strain is HIV-1/HTLV-IIIB.

44. A method for detecting drug resistance of HIV in a sample, comprising:

taking a first cell culture containing CD4 and one or more cell surface co-receptors for HIV at sufficient levels such that productive infection by HIV is achieved;

contacting the first cell culture with a first sample containing HIV;

adding an anti-HIV agent to the first cell culture;

incubating the first culture in the presence of the first sample and the anti-HIV agent for a suitable period time;

taking a second cell culture containing a reporter gene whose expression is regulated by a protein specific to HIV, CD4 and one or more cell surface co-receptors for HIV at sufficient levels such that productive infection by HIV is achieved;

transferring the supernatant of the first cell culture after the incubation to the second cell culture; and

detecting a change in a level of expression of the reporter gene in the cells in the second cell culture.

45. The method according to claim 44, wherein the anti-HIV agent is selected from the group consisting of consisting of nucleoside RT inhibitors, nonnucleoside RT inhibitors, protease inhibitors, integrase inhibitors, viral protein antagonists, capsid lockers, antisense and ribozyme oligonucleotides against HIV mRNA or viral RNA genome, decoys of TAR sequence and RRE, soluble CD4, Gag and Env protein mutants, viral entry inhibitors and fusion inhibitors.

46. The method according to claim 44, wherein the anti-HIV agent is a protease inhibitor.

47. The method according to claim 44, wherein the anti-HIV agent is selected from the group consisting of indinavir, ritonavir, saquinavir, nelfinavir, and amprenavir.

48. The method according to claim 44, wherein the first cell culture is human peripheral blood cells.

49. The method according to claim 44, wherein the first cell culture contains CD4, CXCR4 and CCR5.

50. The method according to claim 44, wherein the second cell culture contains CD4, CXCR4 and CCR5.

51. The method according to claim 44, wherein the reporter gene is selected from the group consisting of genes encoding β-galactosidase, luciferase, β-glucuronidase, chloramphenicol acetyl transferase (CAT), fluorescent protein, secreted embryonic alkaline phosphatase (SEAP), hormones and cytokines.

52. The method according to claim 44, wherein the HIV contained in the first sample is a clinical isolate of HIV.

53. The method according to claim 44, wherein the first sample containing HIV is a blood sample of an individual infected with HIV.

54. The method according to claim 44, wherein the first sample containing HIV is selected from the group consisting of whole blood, blood serum, isolated peripheral blood cells, T cells, spleens, and bone marrow.

52. The method according to claim 44, further comprising:

repeat the steps in claim 44 for a second sample containing a reference HIV strain, and

comparing the change in the level of expression of the reporter gene for the second sample with that for the first sample, wherein an increase in the expression of the reporter gene in the first sample indicates resistance of HIV contained in the first sample to the treatment with the anti-HIV agent.

53. The method according to claim 52, wherein the reference HIV strain is HIV-1/HTLV-IIIB.

54. The method according to claim 44, wherein the level of the cell surface co-receptor for HIV is higher than the corresponding cell surface co-receptor for HIV naturally expressed in a stable human cell line.

55. The method according to claim 54, wherein the human cell line is a human T-lymphoma cell line HUT78.

56. The method according to claim 55, wherein the co-receptor for HIV is CXCR4 or CCR5 and is expressed at a level of at least 2-folds of that of the CXCR4 or CCR5 naturally expressed in a HUT78 cell.

57. The method according to claim 55, wherein the co-receptor for HIV is CXCR4 or CCR5 and is expressed at a level of at least 5-folds of that of the CXCR4 or CCR5 naturally expressed in a HUT78 cell.

58. The method according to claim 44, wherein CD4 receptor and the one or more cell surface co-receptors for HIV contained in the first or second cell culture are expressed by an adenoviral vector tranduced into the cells in the culture.